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Risk factors for dogs becoming rectal carriers of multidrug-resistant *Escherichia coli* during hospitalization

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SUMMARY

This study aimed to identify risk factors for dogs becoming rectal carriers of multidrug-resistant (MDR) *Escherichia coli* while hospitalized in a veterinary teaching hospital. Exposures to potential risk factors, including treatments, hospitalization, and interventions during a 42-day pre-admission period and hospitalization variables, were assessed for 90 cases and 93 controls in a retrospective, risk-based, case-control study. On multivariable analyses, hospitalization for >6 days [odds ratio (OR) 2·91–8·00], treatment with cephalosporins prior to admission (OR 5·04, 95% CI 1·25–20·27), treatment with cephalosporins for >1 day (OR 5·18, 95% CI 1·86–14·41), and treatment with metronidazole (OR 7·17, 95% CI 1·01–50·79) while hospitalized were associated with increased risk of rectal carriage of MDR *E. coli* during hospitalization. The majority of rectal isolates obtained during the study period conformed to MDR *E. coli* clonal groups previously obtained from extraintestinal infections. These results can assist the development of improved infection control guidelines for the management of dogs in veterinary hospitals to prevent the occurrence of nosocomial clinical infections.

Key words: Domestic pets, *E. coli*, epidemiology, nosocomial.

INTRODUCTION

Nosocomial multidrug-resistant (MDR) bacterial infections are becoming more common in veterinary hospitals and the incidence of these infections is expected to increase [1]. Pathogens that have been reported to cause nosocomial infections in dogs and cats include *Acinetobacter* spp., *Escherichia coli*, *Enterobacter* spp., *Enterococcus*, *Klebsiella* spp., *Serratia marcescens* and *Staphylococcus* spp. including methicillin-resistant *S. aureus* [2–8]. The majority of these pathogens are derived from endogenous microbiota of the skin, respiratory and/or gastrointestinal tracts of hospitalized animals or from exogenous sources in the hospital environment, although it is now recognized that veterinary personnel may also play a role in human to animal transmission of nosocomial pathogens [9].

The gastrointestinal tract is the most important reservoir for nosocomial Gram-negative organisms such as MDR *E. coli* and *Enterobacter* spp. and, in humans, intestinal carriage often precedes clinical extraintestinal infection [10]. In humans, the risk of acquiring intestinal colonization or carriage during hospitalization was increased by time spent in intensive-care units [11], disease conditions [12], age [12], urinary and arterial catheterization (probably reflecting manipulation by healthcare personnel) [13, 14], and antimicrobial drug use [11, 12, 15]. In animals, such risk factors remain undefined.

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MDR *E. coli* were isolated from a cluster of nosocomial extraintestinal infections in dogs occurring at a veterinary teaching hospital in Australia [6]. To limit further occurrence of extraintestinal infections, an infection control programme was initiated in which rectal swabs were obtained from dogs on admission for hospitalization and throughout their hospitalization period to isolate MDR coliforms on MacConkey agar containing enoxacin and gentamicin (MCAEG) [16]. Preliminary characterization (pulsed-field gel electrophoresis) of a subset of rectal swab isolates revealed the same two distinct clonal groups of MDR *E. coli* that had been identified from extraintestinal infections, with clonal group 1 corresponding to *E. coli* phylogenetic group A and clonal group 2 to phylogenetic group D [17]. Furthermore, resistance phenotyping and a multiplex PCR test were used to rapidly identify and distinguish between both clonal groups [16].

The infection control programme data from this study provides a unique opportunity to identify specific risk factors that influence gastrointestinal colonization of hospitalized dogs with MDR *E. coli*. In previous work, we identified risk factors for dogs returning a positive rectal swab for MDR *E. coli* on the day of admission to the veterinary teaching hospital [18]. Hospitalization, treatment with a fluoroquinolone, and diagnostic imaging within the 42 days prior to admission increased the risk of carriage of MDR *E. coli* at admission [18]. While identifying dogs which are more likely to introduce MDR pathogens into the hospital environment is an important control strategy, identifying dogs which are at increased risk of developing carriage with MDR *E. coli* during hospitalization is just as important. Therefore, we performed a case-control study using the same infection control programme data to identify risk factors for dogs becoming rectal carriers of MDR *E. coli* while hospitalized. A secondary objective was to compare the clonal group status for rectal isolates obtained during the study period to that of *E. coli* isolates which were causing extraintestinal infections during the same time period [6, 16], using resistance phenotyping and multiplex PCR.

METHODS

Study overview

This study was a retrospective risk-based case-control study using information collected during an infection control programme at The University of Queensland Veterinary Teaching Hospital (UQVTH), a first opinion and referral hospital in Brisbane, Australia, between 7 August 2000 and 15 November 2002. As part of the programme, rectal swabs were collected from dogs on admission to hospital, during hospitalization, and at discharge and were screened for MDR *E. coli* [16]. The unit of interest for this study was the individual admission (where an admission is housing of a dog in the hospital for one or more consecutive nights), and cases and controls were selected at the admission level. Frequencies of exposures to potential risk factors were compared between admissions which became rectal carriers of MDR *E. coli* during hospitalization (cases) and admissions which did not become carriers during hospitalization (controls).

Case and control selection

Study admissions were selected from all admissions to the UQVTH between 1 March 2001 and 30 October 2002. This date range was chosen as compliance with rectal swabbing at admission was the highest during this phase of the infection control programme [18]. Compliance with rectal swabbing procedures during hospitalization was assessed for 1 week of each month. During the 21 weeks from this period that were assessed, 74% of admitted animals were swabbed both during hospitalization and at discharge. During this period, hospitalized dogs were typically swabbed every second day (i.e. median 2 days, range 1–10 days). For the selection of MDR coliforms, rectal swabs were cultured on MacConkey agar containing enoxacin (5 mg/ml) and gentamicin (5 mg/ml) (MCAEG) as previously described [16]. Isolates were stored in Luria–Bertani broth with 15% (v/v) glycerol at $-80\ ^{\circ}C$.

Admissions were eligible for selection if: (*a*) the dog was swabbed on either the day of admission and/or the following day with negative results (no growth on MCAEG), (*b*) at least one more rectal swab was collected on a subsequent day of the same admission and (*c*) the dog was privately owned. Both primary-care and referral admissions were eligible for selection. Most study dogs were swabbed on only one of the first 2 days of hospitalization. All admissions that had at least one positive rectal swab (growth on MCAEG) while hospitalized at any stage after their negative admission swab were selected as cases. Control admissions were matched to the distribution of dates of

admission of cases. For each case admission, one control admission was randomly selected using computer-generated random numbers (generated by the RANDBETWEEN function in Microsoft Excel) from admissions commencing on the same date where the dog had only negative rectal swabs during hospitalization. If there was no admission eligible for selection as a control from admissions commencing on the same date as the case admission, a control was randomly selected from admissions commencing on an adjoining day where available or the next closest day within a week either before or after the case admission. For dogs selected at more than one admission only the first admission within the study period was retained for analysis.

Potential risk-factor data collection

Potential risk factors were selected based on relevant evidence for similar studies conducted in human public hospitals. Data for potential risk factors were collected by examining hospital records as well as the records from referring veterinary practices. We included exposures to potential risk factors from 42 days before admission to start of hospitalization (defined as the pre-admission period) [18]. This time period was selected based on durations of rectal colonization in dogs following experimental infection with MDR *E. coli* [19] (Table 1). Exposures during the hospitalization period were assessed for the period when the dog was at risk of acquiring rectal carriage with MDR *E. coli*; this at-risk period was from admission until either the first positive rectal swab for cases or the last negative rectal swab while hospitalized for control admissions (Table 1). General exposures examined included age, gender, breed, source of admission, and underlying disease or condition (Table 1). Breed was analysed using two categorizing methods: a genetic breed category based on the genetic structure of the purebred domestic dog [20]; and breed group according to the Australian National Kennel Council (ANKC) database [21].

Other putative risk factors could be grouped as hospitalization-specific, non-antimicrobial and antimicrobial-specific treatments, and diagnostic procedures (Table 1). Diagnostic imaging procedures included radiography, echocardiography, ultrasonography, and computer tomography, and other diagnostic procedures included aspirates (chest, joints, wounds), cerebral spinal fluid tap, endoscopy, ear swabs, and tracheal washes.

Data analyses

Associations between potential risk factors and becoming a rectal carrier of MDR *E. coli* were assessed by fitting maximum-likelihood logistic regression models using the LOGISTIC command in Stata v. 10.1 (Stata Corp., USA). Overall significance of each variable was assessed using likelihood ratio test *P* values, and significance of individual levels of risk factors (relative to the reference level) was assessed using Wald *P* values. Potential risk factors with no control admission for one or more levels were assessed using exact logistic regression models, fitted using LogXact 8 (Cytel Inc., USA). For these models, *P* values for each level of the risk factor (relative to the reference level) were calculated as two times the one-sided exact *P* values. Exact probability *P* values were used for hypothesis testing of the overall significance of these risk factors. Odds ratios for these exposure variables were obtained using the median unbiased estimator [22].

Potential risk factors were analysed using both univariable analysis and after adjusting for start date of admission, grouped into 3-month categories. For all factors assessed, the odds ratios changed by $<30\%$ after this adjustment. Accordingly, start date of admission was not fitted into multivariable models and univariable results were used.

This study was taking place at the same time as an infection control programme and this may have altered the risk of acquiring MDR *E. coli* during admissions later in the study period. Therefore, the risk of acquiring MDR *E. coli* was compared between admissions commencing during the first half (1 March 2001 to 30 November 2001) and the second half (1 December 2001 to 18 October 2002) of the study period.

After univariable analysis, all variables with overall likelihood ratio test *P* values $<0{\cdot}2$, other than those requiring analysis using exact models, were examined after adjusting for time at risk of acquiring rectal carriage with MDR *E. coli* while hospitalized.

After adjusting for time at risk while hospitalized, all variables with overall likelihood ratio test *P* values $<0{\cdot}2$ (Supplementary Table 1, available online) other than those with no control admission for one or more levels and those described below were assessed using multivariable modelling. Each of these variables was fitted using a forward selection approach with each variable sequentially fitted in ascending order based on likelihood ratio *P* value. Variables with overall

Table 1. *Putative risk factors for carriage of multidrug-resistant* E. coli *in hospitalized dogs that were assessed in a retrospective, risk-based, case-control study. Exposures to time-varying factors were for the 42-day period prior to admission* (*the* '*pre-admission period*') *and the* '*time at risk while hospitalized*' (*the number of days from admission to first positive swab for cases, or from admission to last negative swab for controls*)

Exposure variable	Exposures during pre-admission period*	Exposures during the pre-admission period and during the time at risk while hospitalized	Exposures during the time at risk while hospitalized
Hospitalization-specific	Duration of hospitalization† Number of times hospitalized Interval between end of final hospitalization and admission† Admission to hospital‡	Ward at UQVTH‡	Time at risk while hospitalized†
Antimicrobial-specific treatments	Interval between the final dose of any antimicrobial§ and admission† Number of antimicrobial§ treatment periods Antimicrobials§ given in hospital or at home	Treatment, number and duration† of treatment with any antimicrobial§ Treatment, duration† of treatment and route of administration (oral or parenteral) of amoxicillin/clavulanic acid and cephalosporins. Treatment with penicillin, fluoroquinolones, metronidiazole, lincospectin, trimethoprim/sulfonamide, imipenem, gentamicin or doxycycline Antimicrobial given after culture and sensitivity testing results§	Interval between admission and treatment†
Non-antimicrobial-specific treatments	Interval between final intravenous fluid treatment and admission† Interval between final NSAID‖ treatment and admission† Interval between final steroid¶ treatment and admission† Interval between final opioid# treatment and admission†	Use and duration† of treatment with intravenous fluids Treatment and duration† with NSAIDs‖ Treatment with steroids¶ Treatment with opioids#	Interval between admission and treatment with intravenous fluids† Interval between admission and treatment with opioids# Interval between admission and treatment with NSAIDs†‖ Interval between admission and treatment with steroids†¶
Diagnostic procedures	None assessed	Urine collection Blood collection Number of general anaesthetics Surgery Number of diagnostic imaging procedures	Other diagnostic tests

NSAIDs, Non-steroidal anti-inflammatory drugs.

* Variables in the first column apply to hospital admissions preceding the index admission.

† Cumulative days hospitalized or treated.

‡ Study dogs were classified as having been admitted to The University of Queensland Veterinary Teaching Hospital (UQVTH), other veterinary hospitals in the surrounding area or a combination of the UQVTH and another veterinary hospital.

§ Antimicrobials included: amoxicillin/clavulanic acid, ampicillin, first-generation cephalosporin, fluoroquinolones (enrofloxacin, orbifloxacin), aminoglycoside (gentamicin), carbapenem (imipenem), lincosamide (clindamycin), metronidazole, tetracycline (doxycycline), trimethoprim/sulfonamide.

‖ Non-steroidal anti-inflammatory drugs (NSAIDs) include carprofen, meloxicam and piroxicam.

¶ Steroidal anti-inflammatories included dexamethasone and prednisolone.

\# Opioids included morphine, fentanyl and buprenorphine.

P values $<0{\cdot}05$ were sequentially excluded before further variables were fitted. Once excluded, variables were not eligible for re-inclusion. Time at risk while hospitalized was forced into all models. The binary variables 'treatment with any antimicrobial in the pre-admission period' and 'treatment with any antimicrobial during time at risk while hospitalized' were not included in the multivariable modelling process as we wanted to assess effects of treatment with particular antimicrobials.

Fit of the final maximum-likelihood logistic model was assessed using the Hosmer–Lemeshow goodness-of-fit test and by comparing observed to expected numbers of cases and controls for ten groups based on predicted probabilities. The discriminatory ability of this model was assessed using the area under the receiver-operating characteristics curve (ROC) and by assessing sensitivity and specificity of the model at varying probability cut-points [23].

Treatment with fluoroquinolones and route of administration of cephalosporins (oral or parenteral) in the pre-admission period, and treatment with gentamicin during time at risk while hospitalized had no control admission for one or more levels and so were further assessed using exact logistic regression by fitting each separately with the variables from the final maximum-likelihood model.

Duration of treatment with any antimicrobial, interval between admission and treatment with any antimicrobial, and the number of antimicrobials during time at risk while hospitalized were further assessed with both univariable analysis and adjusted for time at risk, only for those dogs which were given antimicrobials. Similarly, the route of administration of cephalosporins (oral or parenteral) during time at risk while hospitalized was further assessed only for those dogs which received cephalosporins.

Microbiological characterization of isolates

Microbiological characterization of isolates was performed to compare the rectal *E. coli* isolated during the infection control programme to *E. coli* which was causing extraintestinal infections during the same time period [5, 6] and to extend the observations made by Sidjabat *et al.* [16] regarding carriage of CG1 and CG2 MDR *E. coli* in hospitalized dogs. Case dogs often had more than one positive rectal swab taken, over the duration of their hospitalization. Fifty cases had one positive rectal swab, 21 cases had two positive swabs, 12 cases had three positive swabs, three cases had four positive swabs, and four cases had five or more positive swabs, resulting in a total of 162 *E. coli* isolates. One hundred and thirty-three MDR *E. coli* isolates from the 90 cases were recovered from long-term storage. Disc diffusion susceptibility testing for amoxicillin/clavulanic acid, cefotaxime, cefoxitin, chloramphenicol, enrofloxacin, and spectinomycin was performed using methods described in Clinical and Laboratory Standards Institute (CLSI) guidelines [24, 25]. Isolates were confirmed to be AmpC β-lactamase-producing *E. coli* and were categorized into putative clonal groups based on results of a multiplex PCR for *E. coli uspA*, bla_{CMY} and a class 1 integron-associated *dfra17-aadA5* [16]; isolates positive for all three genes were categorized as MDR *E. coli* putative clonal group 1 and isolates positive for *uspA* and bla_{CMY} only were categorized as putative clonal group 2.

RESULTS

Numbers of cases and controls and underlying disease conditions

In total, 112 admissions met the study selection criteria and all were enrolled as cases but 13 (12%) were subsequently excluded because the dog's clinical case file was missing, leaving 99 assessable case subjects. Clinic files were missing for six (6%) of the 99 control admissions initially selected; these were excluded and replacement control admissions selected. This resulted in 99 case admissions and 99 control admissions from 183 dogs. After retaining only the first admissions for dogs with multiple admissions, 90 case and 93 control admissions were analysed. The underlying disease or conditions for case and control admissions are shown in Supplementary Table 2 (available online). For these control and case admissions, there were, respectively, 137 and 180 intervals between swabs while dogs were at risk of acquiring rectal carriage with MDR *E. coli*. The medians and 90th percentiles of these intervals were 2 and 4 days for both controls and cases. For controls and cases, respectively, 75% and 64% of intervals were 1 or 2 days, 12% and 22% were 3 days, and 13% and 14% were $\geqslant$4 days.

Univariable and multivariable analyses

Associations between potential risk factors and becoming a rectal carrier of MDR *E. coli* were assessed. On univariable analysis, no general risk factors

(age, gender, breed, source of admission, underlying disease or condition) had a P value of $<0{\cdot}2$ (results not shown). Exposures in the pre-admission period, variables which apply to the hospital admission preceding the index admission, with P values of $<0{\cdot}2$ on univariable analysis were: duration of hospitalization ($P=0{\cdot}07$), treatment with any antimicrobial ($P=0{\cdot}18$), treatment with amoxicillin/clavulanic acid ($P=0{\cdot}17$), cephalosporins ($P=0{\cdot}03$), and fluoroquinolones ($P=0{\cdot}06$), route of administration of cephalosporins (oral or parenteral) ($P=0{\cdot}03$), duration of treatment with non-steroidal anti-inflammatory drugs (NSAIDs) ($P=0{\cdot}07$), and surgery ($P=0{\cdot}18$) (Supplementary Table 1).

The risk of becoming a rectal carrier of MDR *E. coli* during time at risk while hospitalized increased markedly with time hospitalized ($P=0{\cdot}02$). On univariable analysis, the following exposures during time at risk while hospitalized were also associated ($P<0{\cdot}2$) with an increased risk of becoming a rectal carrier: housed in intensive-care unit ward ($P=0{\cdot}09$); treatment with any antimicrobial ($P=0{\cdot}04$); duration of treatment with any antimicrobials ($P=0{\cdot}003$); interval between admission and treatment with any antimicrobial ($P=0{\cdot}04$); the number of antimicrobials used ($P=0{\cdot}003$); use and duration of treatment with cephalosporins ($P<0{\cdot}001$); treatment with fluoroquinolones ($P=0{\cdot}02$), metronidazole ($P=0{\cdot}07$), and gentamicin ($P=0{\cdot}01$); route of administration of cephalosporins (oral or parenteral) ($P=0.01$); duration of intravenous fluids ($P=0{\cdot}04$); interval between admission and intravenous fluids ($P=0{\cdot}12$); duration of treatment with NSAIDs ($P=0{\cdot}15$); interval between admission and NSAID treatment ($P=0{\cdot}14$); number of general anaesthetics ($P=0{\cdot}02$); and number of diagnostic imaging procedures ($P=0{\cdot}004$) (Supplementary Table 1).

After adjusting for time at risk while hospitalized, 22 exposure variables remained associated with an increase risk of being a rectal carrier of MDR *E. coli* during hospitalization. In the pre-admission period these included: duration of hospitalization ($P=0{\cdot}05$) treatment with any antimicrobial ($P=0{\cdot}08$); treatment with cephalosporins ($P=0{\cdot}006$), and fluoroquinolones ($P=0{\cdot}04$); the route of administration of cephalosporins (oral or parenteral) ($P=0{\cdot}001$); duration of treatment with NSAIDs ($P=0{\cdot}14$); and surgery ($P=0{\cdot}19$) (Supplementary Table 1).

During time at risk while hospitalized these included: being housed in the intensive-care unit ward ($P=0{\cdot}1$); treatment with any antimicrobial ($P=0{\cdot}004$); duration of treatment with any antimicrobials ($P=0{\cdot}02$); interval between admission and treatment with any antimicrobial ($P=0{\cdot}03$); the number of antimicrobials used ($P=0{\cdot}01$); use and duration of treatment with cephalosporins ($P=0{\cdot}001$); treatment with fluoroquinolones ($P=0{\cdot}028$), metronidazole ($P=0{\cdot}06$), and gentamicin ($P=0{\cdot}03$); route of administration of cephalosporins (oral or parenteral) ($P=0{\cdot}02$); duration of intravenous fluids ($P=0{\cdot}08$); interval between admission and intravenous fluids ($P=0{\cdot}2$); interval between admission and NSAID treatment ($P=0{\cdot}13$); number of general anaesthetics ($P=0{\cdot}04$); and number of diagnostic imaging procedures ($P=0{\cdot}008$) (Supplementary Table 1).

As infection control procedures at the UQVTH were in place during the study, we compared odds of becoming a rectal carrier for admissions in the first and second halves of the study period. On univariable analysis, the odds of becoming a carrier did not differ significantly between admissions in the first and second halves of the study period (OR 0·94, 95% CI 0·53–1·69, $P=0{\cdot}85$).

The final multivariable model consisted of the time at risk while hospitalized; treatment with cephalosporins in the pre-admission period; use and duration of treatment with cephalosporins, treatment with metronidazole, and the number of diagnostic imaging procedures during time at risk while hospitalized; and the interval between admission and treatment with NSAIDs. The result of the final maximum-likelihood logistic model of rectal carriage with MDR *E. coli* in dogs while hospitalized is shown in Table 2.

Treatment with fluoroquinolones and route of administration (oral or parenteral) of cephalosporins in the pre-admission period, and treatment with gentamicin during time at risk while hospitalized, could not be fitted using exact logistic regression modelling with LogXact, possibly due to excessive numbers of zero cells.

The antimicrobial variables: duration of treatment with any antimicrobial; interval between admission and treatment with any antimicrobial; and the number of antimicrobials during time at risk while hospitalized, were not significantly associated with carriage of MDR *E. coli* during hospitalization when only those dogs which were given antimicrobials were included. This indicates that the significant associations between these variables and carriage when all dogs were analysed were probably largely due simply to use of antimicrobials and not to particular durations of

Table 2. *Results of final maximum-likelihood logistic model of rectal carriage with multidrug-resistant* E. coli *in dogs while hospitalized*

Exposure variable	Adjusted OR	95% CI	*P* value
Time at risk while hospitalized (days)*			**0·007**
2–3	Reference level		
4	1·54	(0·51–4·61)	0·442
5	1·88	(0·60–5·88)	0·280
6	2·91	(0·77–11·02)	0·115
7	7·81	(1·95–31·26)	0·004
8–9	4·89	(1·27–18·89)	0·021
>9	8·00	(2·02–39·89)	0·004
Treatment with cephalosporins in pre-admission period			**0·016**
No	Reference level		
Yes	5·04	(1·25–20·27)	0·023
Use and duration of treatment with cephalosporins during time at risk while hospitalized (days)*			**0·004**
0	Reference level		
1	2·05	(0·86–4·87)	0·103
2–12	5·18	(1·86–14·41)	0·002
Treatment with metronidazole during time at risk while hospitalized			**0·034**
No	Reference level		
Yes	7·17	(1·01–50·79)	0·049
Interval between admission and treatment with NSAIDs (days)*†			**0·029**
None	3·64	(1·31–10·07)	0·013
0	Reference level		
1	3·80	(1·14–13·20)	0·030
2	7·69	(1·90–31·15)	0·004
⩾3	2·42	(0·47–12·42)	0·290
Number of diagnostic imaging procedures during time at risk while hospitalized			**0·032**
None	Reference level		
1	0·35	(0·16–0·82)	0·013
⩾2	0·74	(0·22–2·33)	0·599

* Cumulative days, for time at risk, hospitalized or treated.
† Non-steroidal anti-inflammatory drugs (NSAIDs) included carprofen, meloxicam and piroxicam.

use, intervals between admission and treatment, or numbers of antimicrobials used. The route of administration of cephalosporins (parenteral or oral) during time at risk while hospitalized was also not associated with increased risk of MDR *E. coli* carriage during hospitalization when only those dogs which received cephalosporins during time at risk while hospitalized were analysed.

Model fit and discriminatory ability

The final model fitted the data reasonably well with the largest proportional differences between the numbers of observed and expected cases at low and intermediate predicted probabilities. The Hosmer–Lemeshow goodness-of-fit test *P* value was 0·777, providing no basis for concluding that the fit was poor. The discriminatory ability of the final model was fair with the area under the ROC equal to 0·80. At a probability cut-point of 0·48, the model's sensitivity and specificity were both around 0·75.

Characterization of MDR isolates

The antimicrobial disk susceptibility and putative clonal groups of 133 isolates are shown in Table 3.

Table 3. *Putative clonal group and resistance profile for multidrug-resistant* E. coli *isolates from 90 cases (dogs that became carriers) during hospitalization at The University of Queensland Veterinary Teaching Hospital between 1 March 2001 and 30 October 2002*

Putative clonal group*	2001			2002			Resistance profile
	Mar.–May	June–Aug.	Sept.–Nov.	Dec.–Feb.	Mar.–May	June–Oct.	
Clonal group 1	0	6	13	39	6	0	AMC, CTX, FOX, ENR, GEN, CHL
Clonal group 2	6	28	9	8	10	3	AMC, CTX, FOX, ENR, GEN
Other	0	2	2	0	1	0	ENR, GEN†
No isolate‡	2	2	1	0	5	19	n.a.

AMC; Amoxicillin/clavulanic acid, CTX; cefotaxime, FOX; cefoxitin, ENR, enrofloxacin; CHL; chloramphenicol, GEN; gentamicin; n.a., not applicable.

* Putative clonal group 1: positive for *E. coli uspA*, bla_{CMY} and *dfra17-aadA5*. Putative clonal group 2: positive for *uspA* and bla_{CMY} only. Other: all contained *uspA*, 2 contained *dfrA17-aadA5* [16, 17].

† Two of these isolates were also resistant to chloramphenicol.

‡ Isolate non-viable or not stored after original isolation on MCAEG.

Sixty-four (48%) isolates were identified by multiplex PCR as putative clonal group 1 strains (positive for *uspA, dfrA17-aadA5* and bla_{CMY}), and 64 (48%) isolates as putative clonal group 2 strains (positive for *uspA* and bla_{CMY} only). Five (4%) isolates could not be assigned to either of the two clonal groups. These isolates were all identified as *E. coli*; two contained the *dfrA17-aadA5* gene and were possibly clonal group 1 strains that had lost bla_{CMY}, whereas the remaining three isolates may have been clonal group 2 strains that had lost bla_{CMY}, clonal group 1 strains that had lost both the integron and bla_{CMY}, or unrelated isolates. The resistance profiles of CG1 and CG2 MDR *E. coli* isolates are extremely similar and they only differ in their resistance to chloramphenicol. In 93% ($n=84$) of cases, dogs which become rectal carriers of MDR *E. coli*, carried the same putative clonal group throughout hospitalization. However, in six cases, dogs were found to carry a different clonal group at subsequent samplings. In five cases, dogs that initially returned a swab that was positive for putative clonal group 1 were shown to carry a putative clonal group 2 strain on a subsequent swab during hospitalization and in one case; there was a change from putative clonal group 1 to a non-classified group.

DISCUSSION

This study demonstrated that hospitalization for >6 days is an important risk factor for dogs becoming rectal carriers of MDR *E. coli* independently of some antimicrobial treatments. As the duration of hospitalization increases, risk and/or number of contacts with contaminated surfaces and fomites, other hospitalized animals (including MDR *E. coli* carriers), and hospital personnel would also be expected to increase. All are established mechanisms for transmission, gastrointestinal colonization or carriage, and subsequent extraintestinal infection with MDR *E. coli* in humans [10].

Antimicrobial-specific risk factors for dogs becoming rectal carriers of MDR *E. coli* during hospitalization include treatment with cephalosporins in the 42 days prior to admission and treatment with cephalosporins or metronidazole during hospitalization. Treatment with fluoroquinolones prior to hospitalization and treatment with gentamicin during hospitalization were also identified as risk factors for dogs becoming carriers of MDR *E. coli* during hospitalization on univariable analysis.

Antimicrobials suppress susceptible indigenous microbiota [26] and allow other organisms to exploit the vacated ecological niche within the gastrointestinal tract [27]. These organisms could potentially be spontaneous resistant mutants, but given the results of rectal isolate characterization, they are more likely to be MDR *E. coli* that were either ingested following exposure to sources within the hospital, or pre-existing subpopulations normally suppressed to below detectable concentrations by resident microbiota [27]. A range of antimicrobial agents including

cephalosporins, fluoroquinolones, aminoglycosides, and trimethoprim/sulfonamides are significant risk factors for colonization or carriage due to MDR Enterobacteriaceae in hospitalized humans [28–30]. Treatment with fluoroquinolones has been identified as a risk factor for the development of multidrug resistance in rectal *E. coli* in dogs [18, 31].

The most common cephalosporins administered to hospitalized dogs were first-generation cephalosporins. Cefazolin was administered by the parenteral route and cephalexin orally. Cefazolin was often given as one prophylactic injection at the time of surgery. This may explain the lower risk of one day of treatment with cephalosporin during hospitalization compared to more days. In general, parenterally administered cephalosporins are less likely to select for the emergence of resistant Enterobacteriaceae in the intestinal microbiota compared to orally administered cephalosporins [26], even though cephalosporins administered by either route have some suppressive effect on susceptible Enterobacteriaceae and anaerobic bacteria within the gut [26]. However, in this study, there was no difference in risk between the routes of administration of cephalosporins (oral or parenteral).

In this study, all but five of the MDR *E. coli* isolates characterized were confirmed to possess a bla_{CMY} gene. Cephalosporin treatment would certainly provide selection pressure for *E. coli* strains carrying the CMY β-lactamase to be maintained in the gastrointestinal tract of hospitalized dogs. However, co-amoxyclavulanate, a potentiated β-lactam, was the most commonly administered antimicrobial agent, but unexpectedly, it was not found to be associated with MDR *E. coli* rectal carriage. CMY AmpC β-lactamases are resistant to clavulanic acid and experimental colonization or carriage studies [32] will be required to explore the reasons for this key difference.

In the current study, metronidazole and gentamicin treatments were both identified as risk factors for becoming carriers of MDR *E. coli* during hospitalization. However, metronidazole and gentamicin were always administered in combination with other antimicrobials and it is possible that neither treatment increases risk of carriage and the observed associations were due to confounding by other factors, including effects of exposure to other antimicrobials.

Our final multivariable model included interval between admission and treatment with NSAIDs (with lowest risk of carriage in dogs treated from admission start date), and number of diagnostic imaging procedures during hospitalization (with reduced risk in dogs receiving one procedure). We are not aware of biological reasons for such protective effects against the development of MDR *E. coli* carriage during hospitalization but in a previous study, dogs undergoing diagnostic imaging techniques in the pre-admission period were more likely to be carriers of MDR *E. coli* at admission [18]. Unidentified confounding factors probably explain these associations. It is possible that dogs treated with NSAIDs from the date of admission and dogs requiring one diagnostic procedure differed from other dogs. However, underlying disease or condition was not found to be a risk factor for the development of MDR *E. coli* carriage during hospitalization in this study.

The resistance profile and multiplex PCR results generated from rectal isolates demonstrated that the isolates that the case dogs acquired while in hospital were the same as or similar to those isolated from extraintestinal infections [6, 16]. Thus selection pressures are likely to be the same for the emergence of both clonal groups.

The current study had a number of limitations. The selective media (MCAEG) used to isolate may have prevented some MDR *E. coli* from being detected (e.g. fluoroquinolone-resistant *E. coli* strains that carried bla_{CMY} but were gentamicin sensitive), resulting in false negatives.

Diagnostic sensitivity of rectal swabbing in our study may not have been 100%. Sensitivity of rectal swabbing in humans in one study was 90% [32] and in experimental dogs [19] swabbed repeatedly over 21 days, 70% of swabs taken when dogs were known to be carriers were positive for MDR *E. coli* (D. J. Trott, unpublished data). Such errors in admission swabs would have resulted in inappropriate inclusion of dogs who were carriers on admission. If this occurred, the observed odds ratios for each risk factor would reflect the combined effects of that factor on risks of dogs being a carrier and becoming a carrier. False-negative swab results during hospitalization could result in some dogs that became a carrier being incorrectly categorized as controls. For most risk factors, such errors would be expected to be non-differential, i.e. to have occurred with similar frequency in exposed and non-exposed dogs that became carriers. If so, the observed odds ratios for binary exposure variables would be biased towards 1 (i.e. less extreme than actual) and so the true strengths of association would be greater than that indicated by our reported odds ratios.

Rectal swabbing is highly specific for identifying gastrointestinal carriers [26], so there was probably no important bias due to false-positive swab results. Some otherwise eligible admissions were ineligible because they were not swabbed after admission. Because this was due to staff not complying with the hospital swabbing protocol, it is unlikely to have been differential by exposure and case/control status. If so, this would not have been a source of selection bias.

In conclusion, duration of hospitalization, treatment with cephalosporins and metronidiazole during hospitalization, and treatment with cephalosporins prior to hospitalization were important risk factors for dogs acquiring MDR *E. coli* rectal carriage during hospitalization in this study. Partial characterization of rectal isolates confirmed that in almost all cases, the MDR *E. coli* strains were the same or similar to those isolated from clinical extraintestinal infections occurring during the study period. Identification of hospitalized dogs exposed to these risk factors may lead to improved infection control. In addition, risk of acquiring infection could be reduced through prudent antimicrobial use. Although it has been suggested that risk factors for carriage with MDR Enterobacteriaceae may differ from those associated with extraintestinal infection [13], most nosocomial clinical infections are preceded by intestinal colonization or carriage [10, 13]. Therefore, these strategies could reduce the occurrence of MDR clinical infections within large veterinary hospitals when included as part of infection control programmes.

NOTE

Supplementary material accompanies this paper on the Journal's website (http://journals.cambridge.org/hyg).

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Ogeer-Gyles JS, Mathews KA, Boerlin P.** Nosocomial infections and antimicrobial resistance in critical care medicine. *Journal of Veterinary Emergency and Critical Care* 2006; **16**: 1–18.
2. **Glickman LT.** Veterinary nosocomial (hospital-acquired) *Klebsiella* infections. *Journal of the American Veterinary Medical Association* 1981; **179**: 1389–1392.
3. **Boerlin P, *et al.*** Transmission of opportunistic pathogens in a veterinary teaching hospital. *Veterinary Microbiology* 2001; **82**: 347–359.
4. **Sanchez S, *et al.*** Characterization of multidrug-resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. *Journal of Clinical Microbiology* 2002; **40**: 3586–3595.
5. **Gibson JS, *et al.*** Multidrug-resistant *E. coli* and *Enterobacter* extraintestinal infection in 37 dogs. *Journal of Veterinary Internal Medicine* 2008; **22**: 844–850.
6. **Sidjabat HE, *et al.*** Identification of bla_{cmy-7} and associated plasmid-mediated resistance genes in multidrug-resistant *Escherichia coli* isolated from dogs at a veterinary teaching hospital in Australia. *Journal of Antimicrobial Chemotherapy* 2006; **57**: 840–848.
7. **Sidjabat HE, *et al.*** Identification of plasmid-mediated extended-spectrum and AmpC ß-lactamases in *Enterobacter* spp. isolated from dogs. *Journal of Medical Microbiology* 2007; **56**: 426–434.
8. **Scott Weese J.** Antimicrobial resistance in companion animals. *Animal Health Research Reviews* 2008; **9**: 169–176.
9. **Johnson JA.** Nosocomial infections. *Veterinary Clinics of North America Small Animal Practice* 2002; **32**: 1101–1126.
10. **Cookson B.** Clinical significance of emergence of bacterial antimicrobial resistance in the hospital environment. *Journal of Applied Microbiology* 2005; **99**: 989–996.
11. **Filius PM, *et al.*** Colonization and resistance dynamics of Gram-negative bacteria in patients during and after hospitalization. *Antimicrobial Agents and Chemotherapy* 2005; **49**: 2879–2886.
12. **Harris AD, *et al.*** Risk factors for colonization with extended-spectrum beta-lactamase-producing bacteria and intensive care unit admission. *Emerging Infectious Diseases* 2007; **13**: 1144–1149.
13. **Lucet JC, *et al.*** Outbreak of multiple resistant Enterobacteriaceae in an intensive care unit: epidemiology and risk factors for acquisition. *Clinical Infectious Diseases* 1996; **22**: 430–436.
14. **Pena C, *et al.*** Risk factors for faecal carriage of *Klebsiella pneumoniae* producing extended spectrum beta-lactamase (ESBL-KP) in the intensive care unit. *Journal of Hospital Infection* 1997; **35**: 9–16.
15. **Yagci D, *et al.*** Prevalence and risk factors for selection of quinolone-resistant *Escherichia coli* strains in fecal flora of patients receiving quinolone therapy. *Antimicrobial Agents and Chemotherapy* 2009; **53**: 1287–1289.

16. **Sidjabat HE, *et al.*** Emergence and spread of two distinct clonal groups of multidrug-resistant *Escherichia coli* in a veterinary teaching hospital in Australia. *Journal of Medical Microbiology* 2006; **55**: 1125–1134.
17. **Sidjabat HE, *et al.*** Colonisation dynamics and virulence of two clonal groups of multidrug-resistant *Escherichia coli* isolated from dogs. *Microbes and Infection* 2009; **11**: 100–107.
18. **Gibson JS, *et al.*** Risk factors for multidrug-resistant *Escherichia coli* rectal colonization of dogs on admission to a veterinary hospital. *Epidemiology and Infection*. Published online: 15 April 2010. doi: 10.1017/S0950268810000798.
19. **Trott DJ, *et al.*** Canine model for investigating the impact of oral enrofloxacin on commensal coliforms and colonisation with multidrug-resistant *Escherichia coli*. *Journal of Medical Microbiology* 2004; **53**: 1–5.
20. **Parker HG, *et al.*** Genetic structure of the purebred domestic dog. *Science* 2004; **304**: 1160–1164.
21. **ANKC.** Australian National Kennel Council database (http://www.ankc.org.au/home/default.asp). Accessed 10 October 2009.
22. **Hosmer DW, Lemeshow S.** *Applied Logistic Regression*, 2nd edn. New York: John Wiley and Sons, 2000, pp. 336–337.
23. **Dohoo I, Martin W, Stryhn H.** Veterinary epidemiologic research. University of Prince Edward Island, Charlottetown, Prince Edward Island: AVC Inc., 2003, pp. 362–364.
24. **Clinical and Laboratory Standards Institute (CLSI).** Performance standards for antimicrobial susceptibility testing, eighteenth informational supplement. CLSI document M100-S18. Clinical and Laboratory Standards Institute, Wayne, PA, 2008.
25. **Clinical and Laboratory Standards Institute (CLSI).** Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard, 3rd edn. CLSI document M31-A3. Clinical and Laboratory Standards Institute, Wayne, PA, 2008.
26. **Sullivan A, Edlund C, Nord CE.** Effect of antimicrobial agents on the ecological balance of human microflora. *Lancet Infectious Diseases* 2001; **1**: 101–114.
27. **Donskey CJ.** Antibiotic regimens and intestinal colonization with antibiotic-resistant Gram-negative bacilli. *Clinical Infectious Diseases* 2006; **43** (Suppl. 2): S62–69.
28. **Wiener J, *et al.*** Multiple antibiotic-resistant *Klebsiella* and *Escherichia coli* in nursing homes. *Journal of the American Medical Association* 1999; **281**: 517–523.
29. **Asensio A, *et al.*** Outbreak of a multiresistant *Klebsiella pneumoniae* strain in an intensive care unit: antibiotic use as risk factor for colonization and infection. *Clinical Infectious Diseases* 2000; **30**: 55–60.
30. **Graffunder EM, *et al.*** Risk factors associated with extended-spectrum beta-lactamase-producing organisms at a tertiary care hospital. *Journal of Antimicrobial Chemotherapy* 2005; **56**: 139–145.
31. **Ogeer-Gyles J, *et al.*** Development of antimicrobial drug resistance in rectal *Escherichia coli* isolates from dogs hospitalized in an intensive care unit. *Journal of the American Veterinary Medical Association* 2006; **229**: 694–699.
32. **Lautenbach E, *et al.*** Test characteristics of perirectal and rectal swab compared to stool sample for detection of fluoroquinolone-resistant *Escherichia coli* in the gastrointestinal tract. *Antimicrobial Agents and Chemotherapy* 2005; **49**: 798–800.